

## **An optimized method of DNA isolation from highly mucilage-rich okra (*Abelmoschus Esculentus L.*) for PCR analysis**

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### **ABSTRACT**

*In this present study we have developed the protocol for highly purity DNA isolation from the fresh leaves of *Abelmoschus esculentus* and PCR analysis for resultant DNA. Okra is quite difficult to work because of highly acidic polysaccharides (mucilage) and polyphenols present in whole plant, mainly in leaves & fruits. A standard protocol of Doyle & Doyle (1987) was reviewed and modified for DNA extraction from Okra tissues. Modification involved increase the volume of DNA extracting buffer (1.5ml/sample), decrease sample volume (50-60mg), higher salt concentration (5M) and use of polyvinylpyrrolidone employed, yielded a high quality DNA and was found to suitable for PCR and RAPD analysis.*

**Keywords:** genomic DNA, mucilage, okra, polyphenols, random amplified polymorphic DNA.

**Abbreviation:** CTAB, hexadecyltrimethylammonium bromide; PCR, polymerase chain reaction; PVP, polyvinylpyrrolidone; RAPD, random amplified polymorphic DNA.

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### **INTRODUCTION**

Okra (*Abelmoschus esculentus L.*) is cultivated in tropical, subtropical and warm temperate region around the world [1]. Okra is known by many local names in different part of world. It is called 'Lady's finger' in the England, 'Gumbo' in United State of America and 'Bhindi' in India [2]. Okra is said to be very useful against genito-urinary disorders, spermatorrhoea and chronic dysentery [3]. Its medicinal value has been reported in curing ulcers and relief from hemorrhoids [4]. Studies show that daily consumption of 100gm of okra provides 20% of calcium, 15% iron and 50% of vitamin C of humen dietary requirements [5].

Fresh leaves of okra can be obtained from treatment of plant with maiz stover compost used as insecticide in organic farming system in raise okra plant [6]. Previous investigations into the composition and properties of okra mucilage have been reviewed [7]. Various workers have given different composition of mucilage [8, 9, 10]. Okra mucilage to be an acidic polysaccharide consisting of galactose, rhamnose and galacturonic acid [8].

Molecular techniques require isolation of genomic DNA of suitable purity. Due to this reason modified CTAB method provides better DNA extraction suitable for further genomic analysis through molecular markers [11, 12].

Polysaccharides have viscous glue like texture and makes DNA unmanageable in pipetting and unsuitable for PCR since they inhibit *Taq* DNA polymerase activity [13]. Plant contamination like polysaccharides and polypolyphenols compounds are difficult to separate from isolated DNA and these contaminants obstruct polymerases, ligases and

restriction enzymes during their activity [14, 15, 16, 17, 18]. These contaminants are abundance in the foliage of perennials and they co-exist with isolated DNA [19, 20, 21].

In this current study we optimize the DNA isolation protocol for okra and used for RAPD & PCR analysis because okra is highly mucilage and its mucilage interfere in DNA isolation procedure as impurity.

## MATERIALS AND METHODS

### Plant Material:

Fresh young leaves of *Abelmoschus esculentus* collected from different location of Lucknow, Uttar Pradesh were stored at room temperature and used for study. Okra belongs to family Malvaceae and available throughout the year, no difficulty for continual availability of plant material. Leaves and other aerial part are unsuited for storage under low temperature even for short duration due to high mucilage content.

### DNA Extraction and Isolation:

The protocol reported by Doyle & Doyle CTAB method (1987) was employed with following modification: Take 50-60mg of fresh leaves of okra and grinded with liquid nitrogen, after thawing ground the sample in mortar with a preheated CTAB extraction buffer (1.5ml/sample) with 0.2% 2-mercaptoethanol (Table 1). Incubate at 65°C for 1 hour, after that add 1.5µL of RNase and incubate at 37°C for 20 min. Centrifuge at 12000 rpm for 10 min to pellet the debris then add equal volume of Chloroform: Isoamyl alcohol (24:1 v/v) to the supernatant and gently vortex for 10 min and centrifuge 13000 rpm for 10 min. Transfer the supernatant into 0.7 volume of ice-cold isopropanol and 0.15 volume ammonium acetate to precipitate DNA at -20°C for 30 min. Washing done twice by adding 500µL of 70% chilled ethanol to remove ions and then from absolute ethanol. Centrifuge at 13000 rpm for 1 min to pellet the DNA, then air dry and re-suspend in 50µL of TE buffer.

### DNA Quantification

Quantification of DNA first checked by Nanodrop spectrophotometer with absorbance ratio OD [260]/ OD [280] gives the value between 1.7 and 1.9 for all DNA samples. Secondly, electrophoresis was done of all DNA samples on a 0.7 % agarose gel and stained with Ethidium Bromide and view under the UV Transilluminator for quality and yield assessments.

### PCR Amplification Using RAPD Primers

RAPD analysis was performed in five DNA sample with one RAPD primer OPB-7 (Integrated DNA Technologies, Germany). In 25 µL reaction mixture 1x buffer (50mM KCL, 10mM tris-HCL, 1.5mM MgCl<sub>2</sub>), 10mM dNTP's (Sigma), 1.0U *Taq* DNA polymerase (Bangalore Genei), 20pmol primers (IDT, Germany) and 30ng of template DNA to perform PCR reaction in Eppendorf Mastercycler, Germany. Amplification reaction were initiated by 3 min pre-denaturation at 94°C and followed by 35 cycles each at 92°C, 54°C for 1 min, and 72°C for 2 min. A final extension step at 72°C for 10 min was performed after 35 cycles. PCR amplified products were separated by electrophoresis in 2.0% agarose gel at 90 V in 1x TAE buffer. Gel stained with Ethidium Bromide and then was imaged in Alpha DigiDoc (UV-solo model) gel documentation system.

## RESULTS AND DISCUSSION

Isolation of DNA from okra leaves is difficult due to high level of mucilage and polyphenols which interfere with pure DNA isolation. Different degrees of mild and fire type bands appear indicating high level of polysaccharides and polyphenols in standard Doyle & Doyle (1987) method [22] (Fig.1A). In the present study, a modified Doyle and Doyle (1987) protocol yielded good quality DNA. Increased the volume of DNA extraction buffer and decrease the amount of plant material help to remove majority of polysaccharides with high salt concentration [23, 24]. Concentration of 5M NaCl add to the suspension in the lysis step did result in the highest yields of total genomic DNA [25, 26]. Salt use during precipitation of DNA increase solubility of polysaccharides in ethanol thus preventing co-precipitation with DNA [25]. PVP bind effectively to polyphenols compounds which can be separated from DNA by centrifugation [27]. The presence of oxidized phenolic compounds can be reduced further by keeping plant material frozen during homogenization [28]. This is achieved by grinding fresh material in a mortar with under liquid nitrogen. Results from agarose gel electrophoresis show robust and smiling bands of DNA (Fig.1B). The quantity was further estimated by spectrophotometer absorbance ratio OD260/OD280, which varied

between 1.7 and 1.9 for all DNA samples. PCR amplification of DNA obtained from our modified protocol was possible due to absence of impurities (Table 1).

Complete removal of polysaccharides is essential otherwise failure of DNA amplification during PCR due to inhibition of *Taq* DNA polymerase activity [13]. The modified protocol provide high amount of DNA therefore higher content of DNA template for PCR reactions despite the fact that the concentration of total template DNA was slightly higher when using standard protocol. One RAPD primer OPB-7 was tested on five templates DNA. The significant bands appear during amplification of DNA through PCR with RAPD primers was clear as shown in Fig.1C.

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**Table 1: Showing different variation and results for optimization of DNA extraction in *Abelmoschus esculentus***

Doyle & Doyle (1987) Without modification	Doyle & Doyle (1987) With modification	Results
Sample Size: 500mg-1.0 gm	Sample Size: 50-60 mg	Reduce size provide less mucilage
Liquid nitrogen: Not used	Liquid nitrogen: used	Increase surface area of extraction Reduced shearing
CTAB DNA extracting buffer: 1.4M NaCl, 0.02M EDTA, 0.1 M trisHCL (pH 8.0) Per sample volume: Not mention use of polyvinylpyrrolidone: No	CTAB DNA extracting buffer: 5.0M NaCl, 0.5M EDTA, 1 M tris-HCL (pH 8.0) Per sample volume: 1.5ml/sample use of polyvinylpyrrolidone: Yes	Major polysaccharide precipitated and removed in high salt concentration by function of CTAB Key for good precipitation Eliminate protein impurities
Incubation Temp: 60°C for 30 min	Incubation Temp: 65°C for 60 min	Precipitation of major impurities due to increase incubate time
Isopropanol volume used: 0.6 volume	Isopropanol volume used: 0.7 volume	Provide good precipitation of DNA under low temperature
Washing: 76% ethanol	Washing: 70% ethanol, absolute ethanol	Maximum removal of salts & purification of DNA

**Figure 1A: Electrophoresis pattern show mild and fire type bands in the DNA sample isolated using Doyle & Doyle-CTAB method (1987).**

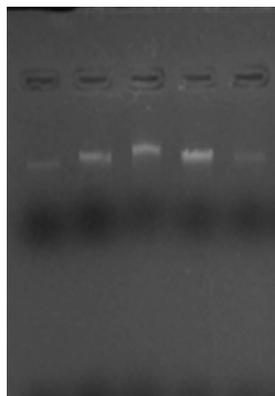


Figure 1B: Clear, sharp, & distinct DNA bands appear during 0.7 agarose gel electrophoresis in currently describe method. Lane 1, 2, 3, 4, 5; DNA band from fresh leaves

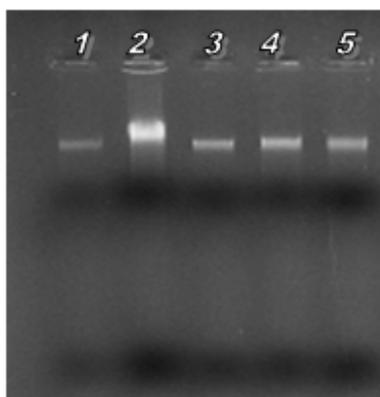
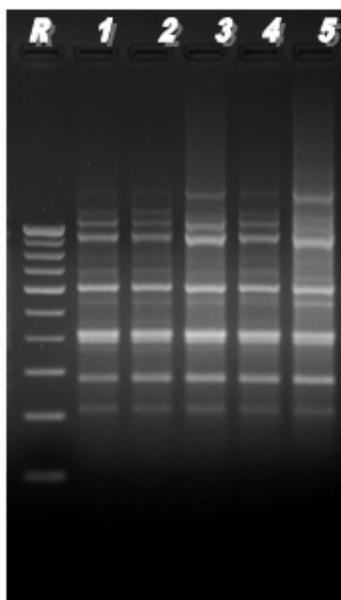


Figure 1C: Analysis of the DNA extracted from *Abelmoschus esculentus* using a RAPD IDT Primers (OPB-7). Lane R; 2KB Ruler, Lane 1, 2, 3, 4, 5; amplified DNA through PCR



### CONCLUSION

Different modification in Doyle & Doyle method helps to remove mucilage and polyphenols in okra leaves for purified DNA suitable for PCR amplification and RAPD analysis. One important modification in given method, use of DNA extraction buffer 1.5ml/sample is key in all modifications. These studies open the door for molecular characterization & genetic improvement works in this promising vegetable and medicinal okra plant.

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